MINIREVIEW

Dynamin Functions and Ligands: Classical Mechanisms Behind

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ABSTRACT

Dynamin is a GTPase that plays a vital role in clathrin-dependent endocytosis and other vesicular trafficking processes by acting as a pair of molecular scissors for newly formed vesicles originating from the plasma membrane. Dynamins and related proteins are important components for the cleavage of clathrin-coated vesicles, phagosomes, and mitochondria. These proteins help in organelle division, viral resistance, and mitochondrial fusion/fission. Dysfunction and mutations in dynamin have been implicated in the pathophysiology of various disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Charcot-Marie-Tooth disease, heart failure, schizophrenia, epilepsy, cancer, dominant optic atrophy, osteoporosis, and Down's syndrome. This review is an attempt to illustrate the dynamin-related mechanisms involved in the above-mentioned disorders and to help medicinal chemists to design novel dynamin ligands, which could be useful in the treatment of dynamin-related disorders.

Introduction

Dynamins were originally discovered in the brain and identified as microtubule binding partners. Dynamin is a 100-kDa protein macromolecule, belonging to the superfamily of GTPases, which plays a major role in synaptic vesicle transport. Members of the dynamin family are found throughout the eukaryotic kingdom. The dynamin family includes dynamin 1 (DNM1), dynamin 2 (DNM2), and dynamin 3 (DNM3), which are known as classic dynamins. Each of these dynamins has the following five different domains: large N-terminal GTPase domain; middle domain (MD); pleckstrin homology domain (PHD); GTPase effector domain (GED); and proline-rich domain (PRD) (Heymann and Hinshaw 2009).

Dynamins, other than classic dynamins, come under the category of dynamin-like proteins, which lacks PHD and PRD and assist in recruiting classic dynamins to cleave the vesicles. It has been observed that membrane fission involves the activities of dynamins and GTP. GTPase-activating proteins facilitate hydrolysis of GTP into GDP with the help of GTPases, whereas guanine nucleotide exchange factors displace the generated GDP, thus favoring the next cycle of hydrolysis. The GTP hydrolysis–dependent conformational change of GTPase dynamin assists in membrane fission, leading to the generation of endocytic vesicles (Praefcke and McMahon, 2004; Ferguson and De Camilli, 2012). Dynamin has been correlated to the pathophysiology of various disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Charcot-Marie-Tooth disease (CMT), heart failure (HF), schizophrenia, epilepsy, cancer, optic atrophy, Down's syndrome (DS), and osteoporosis.

Domains of a Dynamin

Dynamin and GTP together boost membrane fission by GTP hydrolysis and rapid displacement of dynamin from the membrane surface. The distinguishing architectural features that are common to all dynamins and are distinct from other GTPases include the 300-amino acid large GTPase domain or the globular G-domain along with the presence of two additional domains: the MD and the GED. The globular G-domain is composed of a central core that extends from a six-stranded-β-sheet to an eight-stranded one by the addition of 55 amino acids, and this domain is necessary for guanine nucleotide binding, resulting in hydrolysis (Reubold et al., 2005). Two more characteristic sequences of the dynamin

superfamily are MD and a C-terminal GED, which together constitute two distinct domains as stalk and bundle signaling elements (BSEs). The BSE consists of three helices located on the N and C terminus of GTPase domain and the C-terminus of the GED. The BSE conveys nucleotide-dependent conformational changes from the GTPase domain to the stalk and control membrane activity in membrane fission. The stalk is a combination of the MD and the amino-terminal portion of the GED. Depending on the information received from the BSE, the stalk mediates dimerization and tetramerization, and results in the formation of rings and spirals (Wenger et al., 2013). The MD and GED are linked by the PHD, which is limited to classic dynamins and interacts directly with membrane bilayer (Klein et al., 1998; Ramachandran, 2011; Faelber et al., 2012). This domain is highly conserved and essential for dynamin functioning. It helps in binding to the negatively charged head group of phosphatidylinositol-4-5-bisphosphate (PIP$_2$), a membrane lipid that plays a role in clathrin-mediated endocytosis (CME). Mutations in the MD (R361S, R399A) or in the GED (I690K) in human DNM1 result in defective dimerization.

If these mutations occur in the stalk domain, they yield abnormally stable dynamin polymers that are resistant to disassembly and disturb the process of GTP hydrolysis. The PRD has a binding site for the SRC homology 3 domain (SH3D), which is present in various dynamin-related proteins, such as amphiphysin, endophilin, and synaptic dynamin binding protein (syndapin). Thus, amphiphysin, endophilin, and syndapin serve as binding partners of classic dynamins. The PRD is involved in an interaction with SH3D, and, thus, multiple dynamins are engaged in making a network with protein carrying SH3Ds. Various domains of dynamin are given in Fig. 1 (Urrutia et al., 1997; Ford et al., 2011).

Mechanics of Fission: Assembly and Polymerization

Purified dynamin always assembles as a spiral structure, supporting the hypothesis that dynamin wraps around the necks of nascent vesicles. Techniques, like gel filtration and electron microscopy, have found a large–molecular weight helical structure (50-nm wide and a few nanometers long) that is in accordance with the proposed hypothesis (Hinshaw, 2000). When purified dynamins are added to negatively charged liposomes and supplemented with PIP$_2$, they polymerize into helical polymers encircling membrane tubules with increased diameter. On the constriction of the helix, the radius of the neck could be reduced at a level at which the membrane would fuse onto itself and break. Despite lipid membranes being highly flexible, attaining high curvatures requires a large force. Dynamin works against the force, which is generated because of membrane elasticity and lipoidal nature. Lipid bilayers are autosealable; as the pore opens, it spontaneously closes, and only tension higher than 10^{-3} to 10^{-2} N/m can rupture the membrane. These membranes are as resistant to stress as rubber. Obviously, the autosealable property of lipid bilayers makes them difficult to break; thus, seen from a membrane mechanics perspective, membrane fission is far from being a spontaneous process (Pelkmans and Helenius, 2002). Dynamin binds to PIP$_2$ through its PHD and to negatively charged lipids through its positive residues. When a dynamin binds to the

vesicle membrane, the PHD orients toward the inner part of the helix, and polymerization drives membrane flow inside the helix, due to which the membrane gets constrained (50–20 nm) by dynamin coat. The elasticity of the membrane competes with the rigidity of the dynamin coat. The kinetics of dynamin fission depends on bending rigidity, tension, and constriction torque (Morlot et al., 2012).

During polymerization, the force generated is responsible for the deformation of the membrane, which can be measured with optical tweezers using a single-membrane tubule. An in vitro study indicates that dynamin is strong enough to curve the membrane, and the late arrival of dynamin at the curvature shows that it is recruited when the curvature starts forming. Amphiphysin and endophilin are supposed to recruit dynamin at the neck of clathrin-coated pits (CCPs). The assemblage of dynamins at curvatures depends on various factors, such as negatively charged membranes, PIP$_2$, the initial curvature of the membrane, pH level, and salt concentration (Schmid and Frolov, 2011).

Hydrolysis and Conformational Changes

The GTPase domain of dynamin, which is structurally similar to the adenosine triphosphatase domain of kinesin, is responsible for hydrolyzing GTP molecules through GTPase activity (Song et al., 2004). A GTP binding motif known as switch-1 allows the GTPase domain to directly position itself in the most favorable hydrolytic conformation, where positioning depends on interactions with other GTPase domains. Dynamin monomers do not work cooperatively, meaning that each monomer burns its GTP independently. The conformational change associated with GTP hydrolysis has been partially elucidated in the case of dynamin. GTP hydrolysis modulates the helical structure of dynamin, and constriction can reduce the radius of the membrane in its helix, which is opposed by the elasticity of the membrane (Roux et al., 2006). Dynamin generates rotational force during constriction, producing a conformational change that can be evaluated. The required for one turn of dynamin to constrict a membrane tube from a 10-nm radius (the radius of nonconstricted dynamin) to a 5-nm radius (the constricted radius in the presence of GMP-PCP [guanosine-5'-[(β,γ)-methyleno]triphosphate]), by Canham-Helfrich theory, and values close to 500 pN/nm have been found (Lenz et al., 2009; Morlot and Roux, 2013).

Mechanism of Membrane Fission

The mechanism of membrane fission by dynamins has always been a subject of debate and has been analyzed in living cells, broken cells, and artificial lipid bilayers. For fission, the pinches off, poppase, and molecular switch model as three mechanisms have been described. In the pinches off model, dynamin acts as a mechanoenzyme, where it pinches the budding vesicle by hydrolyzing the bound GTP to GDP; whereas, in the poppase model, it stretches like a spring with the help of GTP hydrolysis. In the molecular switch model, dynamin recruits other proteins that trigger the fission (Roux et al., 2006). Dynamin spirals around the neck of the nascent vesicle, and the GTPase domain causes it to constrict by performing a twisting or stretching action that promotes membrane fission, also termed a constriction mechanism. This mechanism is based on the capacity of dynamin to form a self-assembly as a helical polymer around
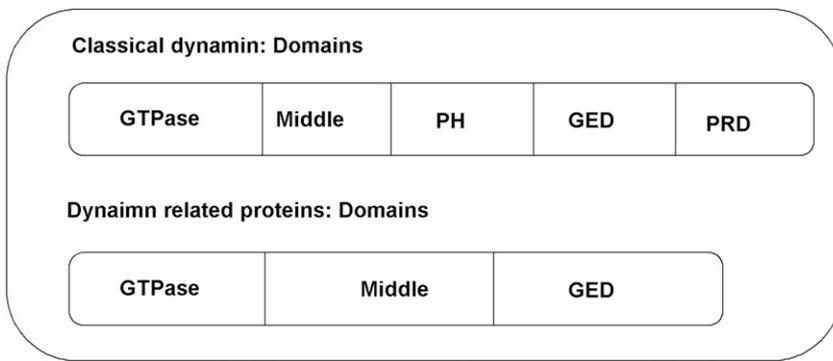
Fig. 1. Domains of dynamin.

the membrane, followed by constriction upon GTP hydrolysis, finally leading to fission. It was suggested that the membrane could be broken by the rapid extension of the helix, tearing off the neck. The spring model relies on the speed of extension (i.e., whether the dynamin helix extends faster than membrane can flow), then the membrane ruptures unless the membrane flows into the cylindrical volume of the helix and adjusts to the new conformation of the polymer without breaking. Thus, fission would occur if constriction were faster than the viscoelastic time of lipid membranes (Danino et al., 2004). This whole process is additionally assisted by actin or myosin motors. Heterogeneous lipid distribution to both sides of the constriction increases the line tension (Lee and De Camilli, 2002). The dynamin helix constriction has been shown by electron microscopy, biochemical, structural, and biophysical data. This constriction is necessary, but not sufficient, for fission, and membrane elastic parameters have an opposite role in constriction (Roux et al., 2010). Other partners, such as actin, which is involved in many fission reactions, could help to control membrane tension. Dynamin has many partners that have a role in membrane remodeling. The future goal is to understand the combined effects of dynamin and its partners involved in fission via constriction (Sweitzer and Hinshaw, 1998; Lenz et al., 2009).

Dynamin and Endocytosis

Endocytosis is characterized by internalization of molecules from the cell surface to the internal cellular compartment. Vesicular trafficking can either be clathrin mediated or clathrin independent. The clathrin pathway is a well-established mechanism of the internalization of pathogens, nutrients, various growth factors, and neurotransmitters. Soluble clathrin from cytoplasm reaches the plasma membrane, where it assembles as a lattice and coats the pits, which are finally pinched off from the plasma membrane with the help of dynamin. Clathrin-binding adaptors, such as adaptor protein-2 bind to cargo vesicles, help in forming a clathrin coat around the vesicles, and mediate endocytosis. PIP_2 also facilitates vesicle formation and budding through epsin, a clathrin adaptor. The coated vesicles fuse with endosomes after endocytosis, and the vesicles are either recycled or degraded by lysosomes. Dynamin-mediated endocytosis process is given in Fig. 2 (Kozlov, 1999; Lenz et al., 2009).

Dynamin interacts with a number of SH3D-containing proteins or dynamin binding partners during the endocytic process through its C-terminal PRD. These dynamin-binding partners are intersectin, amphiphysin, and endophilin (Sundborger and Hinshaw, 2014). Out of binding partners, endophilin controls a

fast-acting tubulovesicular endocytic pathway, which is independent of adaptor protein-2 and clathrin, and is inhibited by inhibitors of dynamin (Boucrot et al., 2015). The existence of the clathrin-independent pathway has been supported by the uptake of, for example, simian virus-40 or interlukin-2 receptor-β into living cells. It could be GTPase dependent or independent (Takei et al., 2005; Mayor and Pagano, 2007; Sundborger and Hinshaw, 2014).

Expression of Dynamin. Transcriptional and translational mechanisms may control the expression of dynamin. The mammalian genome has three genes for dynamin, and the resultant proteins (DNM1, DNM2, and DNM3) have 80% homology. All three dynamins have different expression patterns. Neurons have high levels of DNM1, whereas DNM2 is expressed ubiquitously. DNM3 is expressed in the brain, testes, and lungs. Dynamin and dynamin-related proteins perform a variety of cellular functions, apart from endocytosis (Cao et al., 1998; van der Bliek, 1999).

Binding Partners or Modifiers of Dynamin. Binding partners of dynamins interact with the PRD domain of classic dynamins via the SH3D. Actin binding protein 1 is an example of a binding partner that binds to human DNM2 via the SH3D. Bin/amphiphysin/Rvs domain–containing proteins, such as amphiphysin, endophilin, and syndapin, also interact with the PRD of classic dynamins via SH3Ds and help in the tubulation of lipids (Scaife and Margolis, 1997). Endophilin as a dynamin binding partner binds to the membrane bilayer via its N-terminal region and to both dynamin and synaptojanin (an inositol 5-phosphatase) via its C-terminal SH3D, thus coordinating the function of these proteins in endocytosis. Amphiphysin directs dynamin toward CCPs, where endophilin recruits dynamin to the curvature of the necks of nascent vesicles (Henley et al., 1998; Sundborger et al., 2011). Dynamin modifiers, such as kinases, phosphatase, ubiquitin ligase, small ubiquitin-like modifier (SUMO) ligases and proteases, moderate dynamin activity via complex protein-dynamin interactions. Reversible phosphorylation of human dynamin-related protein 1 (DRP1) at synaptic vesicles occurs via calcium/calmodulin-dependent protein kinase, cyclin-dependent kinase 1, and protein kinase A. Apart from phosphorylation, DRP1 has been shown to undergo SUMOylation, which increases mitochondrial fission (Mishra et al., 2004).

Regulated Activation and Polymerization

Purified dynamin always polymerizes/self-assembles into rings and helices in solutions of suitable ionic strength (Carr and Hinshaw, 1997). Dynamin tubulates membrane bilayers
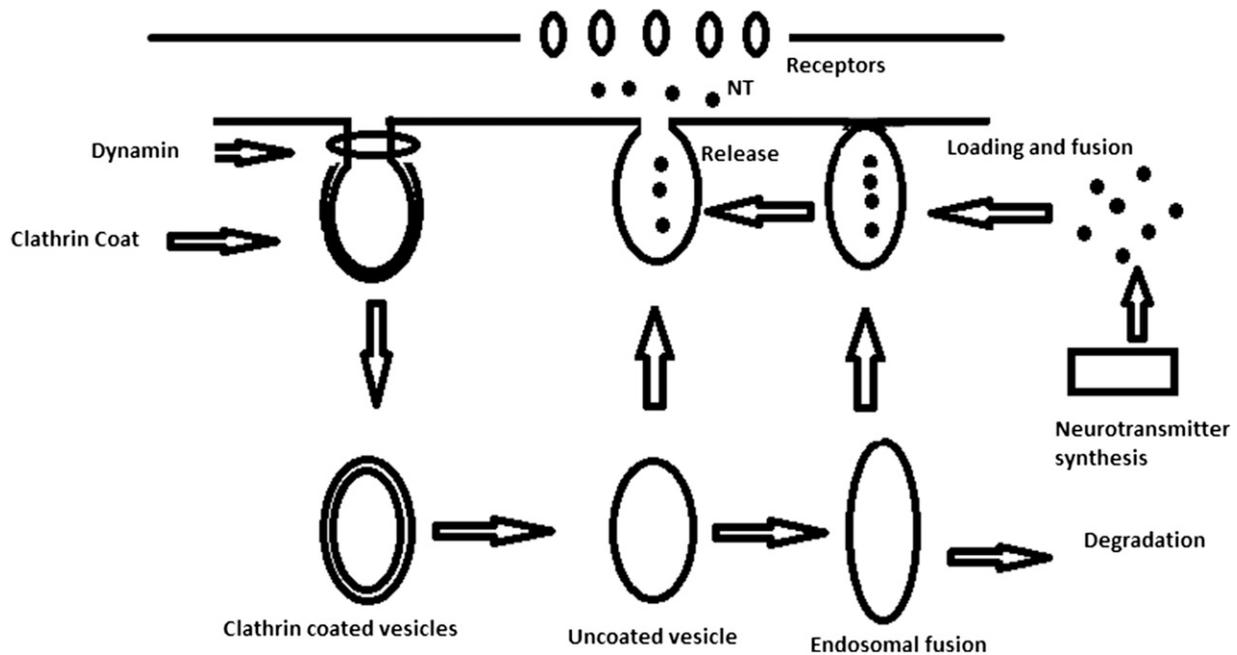
Fig. 2. Dynamin and endocytosis process.

and forms a continuous coat around them. Dynamin polymerization results from the parallel arrangement of dimers at a specific angle, which decides the diameters of the rings. The stalk forms the core of the ring, whereas the BSE and the G domain of the dimer project toward adjacent rungs of the dynamin helix. Dimerization of the GTPase domain is critical for GTP hydrolysis, which can be stimulated during polymerization. Fission could be a result of GTP hydrolysis and membrane destabilization as constricted rings disassemble (Hinshaw and Schmid, 1995).

Dynamin, Defective Mitochondrial Dynamics, and Neurodegenerative Disorders. The role of abnormal mitochondrial dynamics in AD, HD, PD, and various other disorders has been well established. In mitochondrial fission and fusion, Drp1, mitochondrial fission 1 protein, and fusion proteins (Mfn1, Mfn2, and Opa1) are essential to provide ATP to neurons to maintain the normal fission-fusion process. Drp1 is involved in several cellular functions, such as mitochondrial and peroxisomal fragmentation, SUMOylation, phosphorylation, ubiquitination, and cell death. In neurodegenerative diseases, including AD, PD, HD, and amyotrophic lateral sclerosis, mutant proteins interact with Drp1 and activate mitochondrial fission machinery. This activation leads to excessive mitochondrial fragmentation and impairs mitochondrial dynamics, which finally causes mitochondrial dysfunction and neuronal damage (Reddy et al., 2011).

Advancements in molecular biology and genetic analysis have revealed that mutations of human DNM1 and DNM2 are very well linked to various disorders, such as AD, PD, HD, CMT, HF, schizophrenia, epilepsy, cancer, and optic atrophy. CMT results from a defect in the PHD of dynamin that leads to defective lipid binding. Defects in the MD and PHD are very well linked to centronuclear myopathy (CNM).

Dynamin in AD

AD is the most prevalent age-related disorder characterized by neurodegeneration and cognitive decline. Synaptic dysfunction is one of the most important events in the pathogenesis of AD (Kelly et al., 2005). The causes include accumulation of amyloid-β (Aβ) protein, phosphorylated tau, and neurofibrillary tangles in the brain. DNM1 is involved in the regulation of amyloid generation through the modulation of BACE1. It reduces both secreted and intracellular Aβ levels in cell culture.

Aβ and phosphorylated tau interact with Drp1, the mitochondrial fission protein, and cause excessive fragmentation of mitochondria, which leads to abnormal mitochondrial dynamics and synaptic degeneration in neurons, which are responsible for AD (Kandimalla and Reddy, 2016).

The interaction of Aβ and Drp1 initiates mitochondrial fragmentation in AD neurons, and abnormal interaction increases with disease progression (Manczak et al., 2011). Aβ protein causes synaptic disturbances, which result in neuronal death (Zhu et al., 2012).

The down-regulation of DNM2 has also been linked to Aβ in hippocampal neurons. The dynamin binding protein gene, located on chromosome 10, is associated with late-onset AD (LOAD) (Kuwano et al., 2006; Aidaralieva et al., 2008). The connection between DNM2 and LOAD is not clear, but a decreased expression of hippocampal DNM2 mRNA has been found in LOAD. DNM2 dysfunction affects metabolism and localization of the Aβ protein and amyloid precursor protein (APP). Real-time PCR analysis showed that the amount of DNM2 mRNA was significantly lower in the temporal cortex of AD brains and the peripheral blood of dementia patients compared with that of the control. However, DNM1 and DNM3 were not significantly affected. Analysis of peripheral leukocyte in dementia patients showed that levels of DNM2 were significantly lower than those of the control. Hence, it was assumed that reduced levels of DNM2 mRNA caused dysfunction in DNM2 (Aidaralieva et al., 2008; Kamagata et al., 2009). Research has been done to investigate the relationship between DNM2 dysfunction and Aβ production, which is a key event in AD pathology. Neuroblastoma cells with dominant-negative

DNM2 resulted in Aβ protein (Ab) secretion, and most of the APP in these cells was localized to the plasma membrane. An accumulation of APP near plasma membrane shows DNM2 dysfunction, which is normally transported via endoplasmic reticulum and Golgi apparatus to the plasma membrane is and finally taken up by endosomes via endocytosis for Ab generations (Carey et al., 2005). The lipid raft is also a major source of Ab generation, and the plasma membranes of DNM2-negative neuronal cells have been found with an increase in the concentration of the lipid raft. An increased amount of flotillin-1, a marker of lipid raft, was found to be in the plasma membrane of DNM2-negative neuronal cells, confirming the increased presence of lipid raft (Meister et al., 2014). DNM1 knockout mice show reduced levels of secreted and intracellular levels of Aβ in cell cultures. A dramatic reduction in beta-site APP-cleaving enzyme 1 (BACE-1) cleavage products of APP has been found in DNM1 knockout mice. A decrease in Ab with DNM1 and BACE1 inhibitors does not show a combined effect, which indicates that the effects of DNM1 inhibition are mediated through the regulation of BACE1. Role of dynamin in Alzheimer's disease is given in Fig. 3 (Sinjoanu et al., 2008).

Epilepsy

An epileptic seizure is characterized by social, cognitive, and psychological impairment. It results from abnormal neuronal discharge originating from the brain (Singh and Jadhav, 2014). Synaptic transmission is an important communication between presynaptic and postsynaptic neurons and depends on the formation, release, and endocytosis of the synaptic vesicle. Abnormalities in synaptic transmission are responsible for various neurologic disorders, including epilepsy.

Upregulation of DNM1 has been correlated in some epilepsy patients (Li et al., 2015). Dynamin, synapsin and syndapins are involved in vesicle formation, neurotransmitter release, and recycling of neurotransmitter, which binds to postsynaptic receptors (i.e., inhibitory GABA receptors and to the excitatory glutamate receptors. The activation of glutamate receptor further activates a variety of postsynaptic receptors, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, N-methyl-D-aspartate, kainite, and metabotropic receptors. The activation of the receptors triggers various signaling cascades and results in a vast array of effects, which can be modulated by numerous auxiliary regulatory subunits. Different neuropeptides, such as neuropeptide Y, brain-derived neurotrophic factor, somatostatin, ghrelin, and galanin, also act as regulators of diverse synaptic functions (Casillas-Espinosa et al., 2012). There is no direct evidence linking dynamins to epilepsy, but electrophysiological responses from neurons of mutant mice show defects in GABAergic neurotransmission, which are similar to those of dynamin-1 knockout mice. Missense mutations in dynamin1 have been found to cause epilepsy in the fitful mouse model (Boumil et al., 2010). Perturbations of dynamin-1 function can enhance susceptibility to seizures. The inhibition of dynamin binding to syndapin with a peptide based inhibitor slows down the abnormal neuronal firing. Syndapin knockout mice show high impairment in the recruitment of dynamin to the nascent vesicle and suffer from seizures (Koch et al., 2011). Synapsin 1, 2, and 3 or phosphoprotein interact with synaptic vesicles and prevent them from being trafficked to presynaptic membrane. During action potential generation, they are phosphorylated and allow vesicles to release neurotransmitter. Mutations in synapsin-1 and the inhibition of dynamin also alter the process and have been linked with abnormal neuronal discharge (Newton et al., 2006). Thus, there is a need to search legends that inhibit abnormal neuronal discharge through endocytosis and at the same time allow normal neuronal activity to occur (Anggono et al., 2006; Baldelli et al., 2007).

HD

HD is a genetic autosomal-dominant neurodegenerative disease, caused by the expansion of a CAG repeat in the huntingtin gene and results in ataxia, chorea, and dementia as
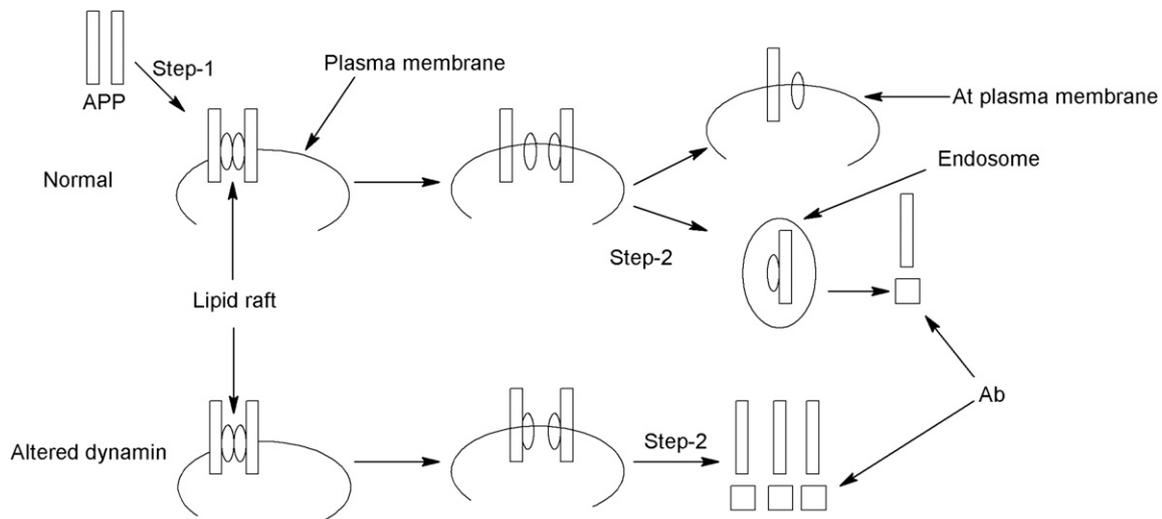
Fig. 3. Dynamin and Alzheimer's. Step-1: Transport of APP to plasma membrane through the endoplasmic reticulum and the Golgi apparatus. Normal process, step-2: It is taken up by endosomes where Aβ generation occurs. Altered process, step-2: DNM2 negative neuronal cells have accumulation of APP in the plasma membrane, because the major role of DNM is in endocytosis. In DNM dominant negative neuronal cells, increased Aβ production may occur due to the accumulation of APP and the presence of lipid raft.

major symptoms (Bates et al., 2015). HD was first described by George Huntington in 1872. Dysfunction of the Huntington protein (Htt) and disturbances in the mitochondrial electron transport chain are supposed to be the main causes of HD (Zeviani and Carelli, 2005). Mitochondrion is one of the organelles whose electron transport chain, calcium buffering capacity and morphology is severely affected in HD. In recent years, biochemical and genetic studies have shown that there is a link between Htt and clathrin-mediated endocytosis (Harjes and Wanker, 2003; Chakraborty et al., 2014). The cell death process in HD is initiated in the mitochondria and Htt aggregates are found in their vicinity. Recently, mutant Htt (mHtt) has been shown to affect the activity of Drp1 via SUMOylation and nitrosylation, resulting in excessive endocytic fission (Otera et al., 2013). The increase in Drp1, fission 1 protein, and cyclophylin D and the decrease in Mfn1 and Mfn2 have been linked with abnormal mitochondrial dynamics in the cortex of HD patients. The presence of mHtt oligomers in HD neurons and mitochondria may affect normal neuronal functions. In the affected brain regions of HD patients, mHtt in association with impaired mitochondrial dynamics alters the axonal transport of mitochondria and results in decreased mitochondrial function and damaged neurons (Shirendeb et al., 2011). The cortex and cerebellum in the brain of the HD patient show a stage-dependent increase in DRP1. DRP1 is a substrate for mitochondrial ubiquitin ligase and may affect the activity of the ubiquitin proteasomal system, and the dysfunction of the cellular ubiquitin proteasomal system has been found to be the main reason for increased mHtt aggregate formation, which is a result of reduced mitochondrial electron transport chain complex III. Recent studies (Shirendeb et al., 2011; Reddy, 2014) have found that mHtt interacts with Drp1, causes excessive fragmentation of mitochondria, and is associated with impaired transmission. Role of DRP1 in mitochondrial fission/fusion is given in Fig. 4.

Mitochondrial fission or division is controlled DRP1, and the translocation of DRP 1 to mitochondria is regulated by its GTPase activity, phosphorylation, SUMOylation, and nitrosylation. mHtt is now known to increase GTPase activity and nitrosylation, thereby increasing DRP1 affinity to mitochondria, and results in an enhanced mitochondrial fission process.

CMT and CNM

This monogenetic disease is characterized by impaired motor and sensory neuronal functions, resulting in muscle weakness and foot ulcers leading to frequent infections. The disease is a result of mutations of genes associated with intracellular trafficking (Szigeti and Lupski, 2009; Durieux et al., 2010). Mutations in DNM2 result in CMT. These mutations are mainly clustered in the N-terminal part of the PHD of dynamin, which is involved in interactions with phosphoinositides and results in dominant intermediate CMT type 2B (Sidiropoulos et al., 2012). Interestingly, mutations in myotubularin-related protein 2, which is responsible for the metabolism of phosphatidylinositol bisphosphate and phosphatidylinositol 3 phosphate, leads to different types of CMTs, confirming the role of synaptic transport in CMT. CME is necessary for proper myelination, and defects in this function are caused by CMT mutants; thus, CMT mutants are major contributors of the pathology of CMT subtype 2B (Koutsopoulos et al., 2011). Defects in CME result in improper myelination in CMT-associated mutants.
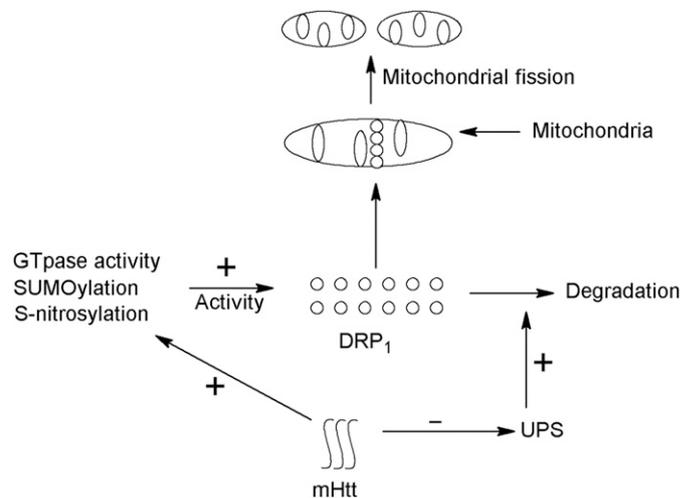
Fig. 4. DRP1 and mitochondrial fission: Mitochondrial fission or division is controlled DRP1 and Translocation of DRP 1 to mitochondria is regulated by its GTPase activity, phosphorylation, SUMOylation, and nitrosylation. mHtt is now known to increase GTPase activity and nitrosylation, thereby increasing DRP1 affinity to mitochondria, and results in enhanced mitochondrial fission process.

The dependency of myelination on DNM2 and CME gives a hint that CME could be a new target for CMT treatment. Mutations in DNM2 lead to dominant intermediate CMT type B, and other mutations in DNM2 cause autosomal-dominant CNM (Sidiropoulos et al., 2012). DNM2-related CNM is a slowly progressive, rare, inherited disorder, which is accompanied by a general facial muscle weakness, ptosis, and extraocular muscle palsy. Cognitive impairment has also been reported in some CNM patients. It was speculated that DNM2 mutants would cause CNM by interfering through centrosomes. Mutations in the C-terminal α-helix of PHD appear to cause conformational changes in dynamin and to affect the GTP hydrolysis cycle. DNM2 mutations affecting the MD and the PHD have been identified in autosomal-dominant CNM (Bitoun et al., 2009; Kenniston and Lemmon, 2010). In a patient-based study (Echaniz-Laguna et al., 2013), DNM2 mutations were found to be the major cause of CNM, but the molecular mechanisms that lead to neuropathy and myopathy need to be explored further.

PD

PD has been known to be associated with tremors, slow movement, and cognitive difficulties. Amphiphysin and endophilin are two Bin/amphiphysin/Rvs proteins that bind to dynamin, where amphiphysin targets dynamin to CCPs and endophilin directs dynamin to the necks of the nascent CCPs. There is a link between the PD gene Parkin and endocytic protein endophilin. Parkin performs degradation of DRP1, and mutation leads to the accumulation of DRP1 for mitochondrial fragmentation. A hypothesis proposed that endophilin helps in recruiting Parkin at endocytic pathways to prevent or regulate the degradation of synaptic proteins. Mutations of Parkin, an E3 ubiquitin protein ligase, lead to autosomal juvenile-onset of PD. Endophilin binds to the Ubi domain of Parkin via the SH3D and is said to become ubiquitinated (Wang et al., 2011; Stafa et al., 2014). Parkin levels significantly increase in the brain and fibroblasts of endophilin mutant mice (Cao et al., 2014).

The absence of endophilin or synaptojanin knockout results in a robust increase of Parkin in the brain. Endophilin-Parkin interactions may affect the synaptic vesicle transmission and might be involved in the pathogenesis of PD. Drp1 is a regulator of mitochondrial fission and is found to be reduced in wild-type DJ-1 cells and increased in mutant DJ-1 cells. DRP1 knockdown in these mutant DJ-1 cells restores the normal mitochondria morphology. DJ-1 is involved in the regulation of mitochondrial dynamics through the modulation of dynamin-like protein 1/DRP expression. PD-associated DJ-1 mutations may cause PD by impairing mitochondrial dynamics and function through DRP (Hu et al., 2012). Mutations in leucine-rich repeat kinase 2 (LRRK2) and interactions of LRRK2 with endophilin, further interactions of endophilin with Parkin, are probable causes of autosomal familial PD. LRRK2 is involved in synaptic vesicle endocytosis and exocytosis, and it has been linked with DNM1, DNM2, and DNM3. LRRK2 also interacts with dynamin-related proteins, which are involved in mitochondrial fission and fusion (Smith et al., 2005; Cao et al., 2014).

Cancer

Altered mitochondrial functions are also associated with cancer. Targeting mitochondria for the restoration of normal functioning or insisting mitochondria-induced cell death are some important strategies for cancer treatment. Drp1 has been found to be up-regulated in certain types of cancers, such as lung and breast cancer. A further role of Drp1 in cell migration and apoptosis in cancer cells has been linked recently, uncovering Drp1-mediated mitochondrial fission as an effective therapy for cancer (Frank et al., 2001; Qian et al., 2013). Prostate cancer (PCA) is the second most fatal cancer in men, although the mortality rate has been reduced significantly in recent years. Significantly increased expression of DNM2 has been found in advanced stages of progressive PCA compared with the starting stage, although the importance of expression is largely unknown. In some preclinical studies, suppression of the DNM2 gene significantly reduces cell migration and tumor size, both in vitro and in vivo, respectively. The conclusion is that overexpression of DNM2 is associated with neoplastic prostate epithelium and is a potential target for PCA. DNM2 is essential for endocytosis of some proteins associated with cancer motility and invasiveness, such as integrin-β1 and focal adhesion kinase. Overexpression of DNM2 in PCA and the requirement for DNM2 in endocytosis of focal adhesion kinase and integrin open the gate for a new therapy that can control the expression of DNM2 (Xu et al., 2014). In hepatocellular carcinoma, DNM3 has been a candidate tumor suppressor gene (Inokawa et al., 2013).

Depletion of endogenous Dyn2 inhibited platelet-derived growth factor receptor α (PDGFRα)–stimulated phosphorylation of Akt and Erk1/2. Tyrosine-protein phosphatase, a non-receptor type-2 (SHP-2), interacts with Dyn2 and PDGFRα signaling. Dyn2 mediates PDGFRα-SHP-2–induced glioma tumor growth and invasion, suggesting that targeting the PDGFRα-SHP-2-Dyn2 pathway could give a new hope to patients with malignant glioblastomas (Feng et al., 2012). Current treatments for glioblastomas include nitrosoureas, cisplatin, irinotecan, gefitinib, and erlotinib. Findings from the Children's Medical Research Institute correlate the role of DNM2 in the treatment of glioblastoma. Since DNM2 plays a role in the final stage of mitosis and cytokinesis, inhibitors of DNM2 could help to treat glioblastoma.

Dominant Optic Atrophy

Optic nerve fibers are responsible for carrying image information from the retina to the brain. A defect in any fiber can impair vision because of the disruption of impulses being sent to the brain. This abnormal condition is known as optic atrophy. The patient complains of having blurred vision and trouble with peripheral and color vision. A gradual loss of visual activity is observed, which often leads to blindness (Lenaers et al., 2012). Optic atrophy is linked to OPA1, which is a GTPase of the dynamin family that is present in the inner mitochondrial membrane and is hypothesized to be involved in mitochondrial fission. Forty-five percent of existing cases of dominated optic dystrophy are said to arise from a mutation of OPA1 (Delettre et al., 2000). Mitochondrial network dynamics, fission, and fusion mediate mitochondrial quality control. Proteolytic cleavage of OPA1 prevents mitochondrial fusion. The OPA1 long isoform counteracts cytochrome C release and hence acts as an antiapoptotic. An OPA1 mutant affects mitochondrial quality control, rendering cells susceptible to stress, especially retinal ganglionic cells, and the risks to retinal ganglionic cells are very well linked to glaucoma and dominant optic atrophy. OPA1 polymorphisms have been associated with certain forms of glaucoma (Alavi and Fuhrmann, 2013).

Schizophrenia

Schizophrenia is a devastating mental disorder that is characterized by a breakdown in thinking and poor emotional response. Neuropathological evidence suggests that dopaminergic, GABAergic, and glutamatergic transmissions are involved in the symptomatology of schizophrenia. Dystrobrevin-binding protein 1 or the dysbindin gene located on chromosome 6p has been linked to the etiology of schizophrenia. It affects neurotransmission and is responsible for cognitive dysfunctions associated with schizophrenia. The expression level of dysbindin is found to be reduced in the hippocampus and prefrontal cortex of schizophrenia patients (Numakawa et al., 2004). Dysbindin protein expression also affects the levels of dopamine and glutamate in the hippocampus. Studies support the role of CME in the pathophysiology of schizophrenia and bipolar disorders. Dysbindin is involved in processes closely related to CME and membrane trafficking (Chen et al., 2008; Schubert et al., 2012). Dysbindin deficiency is responsible for "dysbindin-like defects," such as slow fusion kinetics, decreased neurotransmitter release, and reduced small readily releasable pool. Finally, synaptic neurotransmission is affected in schizophrenia (Feng et al., 2008; Dickman and Davis, 2011). Further, antipsychotic drugs have been found to interact with clathrin-interacting proteins. Thus, the involvement of dysbindin in membrane trafficking and the interaction of antipsychotic medicines with clathrin protein indicate a new approach to be explored in schizophrenia.

HF

Cardiac mitochondria serve as major source of energy and radicals and are important for normal functioning of the heart. There has always been a link between mitochondrial number and

structure, and mitochondrial fusion and fission, including mitophagy. Left ventricle (LV) ejection fraction failure or LV HF, hypertension, and idiopathic cardiomyopathies are various etiologies of HF (Palaniyandi et al., 2010). Mitochondria are dynamic cell organelles that keep on dividing and fusing to maintain their number and integrity (Murray et al., 2007). Abnormal mitochondrial morphologies have been linked to many cardiac diseases, strongly suggesting that mitochondrial fusion and fission are required for normal functioning of the heart. The disruption of Drp 1 leads to mitochondrial elongation and inhibition of mitochondrial autophagy, inducing mitochondrial dysfunction, which causes cardiac dysfunction (Ikeda et al., 2015).

Drp1 activation during ischemia reperfusion results in LV dysfunction, implying that Drp1 inhibition is beneficial for heart activity (Sharp et al., 2014). Cardiac myopathy is linked to a decreased level of OPA1. The protein OPA1 is a GTPase of the dynamin family and is present in the inner mitochondrial membrane. It is expected to be involved in mitochondrial fission. Studies in rats having HF found that are a reduced number of mitochondria and structural changes, such as disorganized cristae/reduced cristae density. This provides evidence that Drp1 induced fission could further be explored for HF (Chen et al., 2009).

Osteoporosis

Pyrophosphates have long been identified as the first choice of treatment for osteoporosis. These drugs are believed to inhibit prenylation and to disrupt the signaling pathways downstream of prenylated small GTPase (Wark, 1996). Prenylation inhibitors are found to be antiviral agents, and investigation has shown that a prenylation-independent pathway can also suppress viral infections. Recently, it has been found that bisphosphonates target DNM2 by inhibiting their GTPase activity, thereby blocking the endocytosis of adenovirus. Thus, by inhibiting dynamin-mediated endocytosis, the bisphosphonate class of drugs provides a new strategy for the treatment of osteoporosis (Masaike et al., 2010).

DS

DS or trisomy 21 is a condition in which a person is born with extragenetic material from chromosome 21 that causes, learning, language, and memory disabilities. Overexpression of the regulator of calcineurin 1, an endogenous calcineurin inhibitor, affects calcineurin phosphatase signaling, leading to DS (Kuruvilla et al., 2004). Activity-dependent bulk endocytosis (ADBE) is one of the mechanisms by which synaptic vesicles reform. ADBE couples neuronal activity, and calcineurin causes dephosphorylation of DNM1 so that it binds with syndapin. In simpler words, calcineurin modifies DNM1 and stimulates ADBE. The disruption of the calcineurin-DNM1 interaction inhibits CME. It has been proved that all downstream defects in brain function in DS are due to the dysfunction of DNM1 (Lai et al., 1999). The up-regulation of regulator of calcineurin 1 and dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A results in the dysfunction of DNM1 phosphorylation and gives rise to defective ADBE (Fuentes et al., 2000; Clayton and Cousin, 2009).

Nephrosis

The function of Bowman's capsule, in the kidney, is to filter blood by allowing small molecules, such as salts, sugar, and water, to pass through and retaining beneficial macromolecules, such as proteins. Podocytes are visceral epithelial cells of Bowman's capsule that wrap around capillaries of the glomerulus. Bis-T-23, a small molecule, promotes actin-dependent dynamin oligomerization and increases actin polymerization in injured podocytes, which results in improved renal health in diverse models of both transient kidney disease and chronic kidney disease (Schiffer et al., 2015). There has been a link between the mechanism that governs podocyte processes and neuronal synapse development. Dynamins act on actin filaments of cytoskeleton of podocytes and help in the development of a podocyte network, after which podocyte foot process levels of DNM1 and DNM2 are suppressed (Soda et al., 2012). The disruption of this sequence of events causes nephrotic syndrome.

Ligands of Dynamin

Dynasore. The inhibition of dynamin reversibly blocks synaptic vesicle endocytosis. Dynasore rapidly inhibits the GTPase activity of dynamin with high specificity without disturbing exocytosis. In the presence of dynasore, a stimulation of weak frequency can cause the accumulation of vesicular proteins on the cell surface, even after stimulation is terminated. This shows that the events of endocytosis rely on dynamin and that dynasore successfully inhibits these events. It has been further proved by ultrastructural analysis that dynasore causes a reduction in the density of synaptic vesicles. Macia et al. (2006) proposed a dual role of dynamin in endocytosis (i.e., one during detachment of the vesicle and a second at the time of invagination). They screened 16,000 compounds and came up with dynasore, which interfered with the in vitro activity of DNM1, DNM2, and DRP1. Dynasore has the ability to block the GTPase activity of dynamin. It noncompetitively inhibits basal and stimulated rates of GTP hydrolysis without changing GTP binding. In cultured cells, it blocks clathrin-mediated endocytosis completely. Dynasore acts as a potent inhibitor of endocytosis by rapidly blocking the formation of coated vesicles and is supported by half-formed and O- and U-shaped pit formation. It acts as a noncompetitive inhibitor of the GTPase activity of DNM1 and DNM2. Dynasore interferes with all functions of dynamin in endocytosis, such as low-density lipoprotein and transferrin transport, which happens through CME. Transferrin uptake was well blocked by the pretreatment of cells with dynasore. It does not affect the functions that are independent of dynamin. The action of dynasore is very fast, and it depends on diffusion of the molecule to coated pits in the required concentration (Macia et al., 2006; Nankoe and Sever, 2006; McGeachie et al., 2013).

Other Ligands

Lee et al. (2010) have synthesized 2-naphthohydrazides, 2-naphthoamides (Fig. 5), and naphthoates, which show potent inhibition of CME compared with dynasore. The starting materials for these compounds are substituted 3-hydroxynaphthoic acids and are available commercially. A carboxylic acid group of these starting materials was converted to esters via Fischer esterification reaction. The
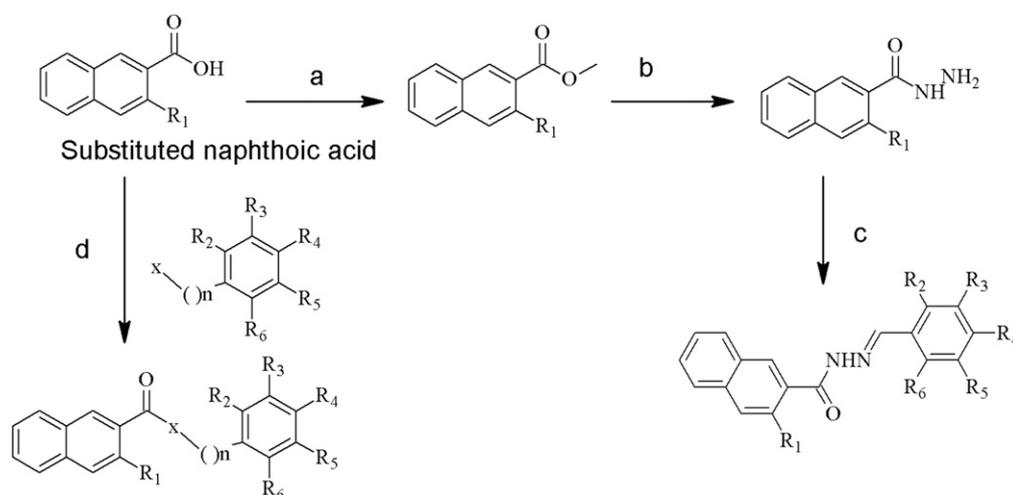
Fig. 5. Synthesis scheme for compound naphthohydrazide and naphthoamides. Reagent and conditions (a) HCl, MeOH, 1 h at 0°C, 1h at RT; (b) hydrazine monohydrate, EtOH, 24h, 20°C; (c) ceric ammonium nitrate, EtOH, 3h, 65°C; (d) DMAP, DCI, DCM, 5h at RT.

resultant ester was substituted with hydrazine hydrate in ethanol to get hydrazide. Further derivatives were obtained by the reaction of hydrazide with various substituted aldehydes. For amide compounds, activated napthoic acid was reacted with various amine compounds in desired conditions. DD-6 (R1,R2, R3,R6 = H,R4,R5 = OH) and DD-11 (R1,R3 = OH) compounds more potently inhibit membrane fission. The introduction of chlorine or dimethyl substitution on the phenyl ring abolishes the inhibitory activity of dynasore. An hydroxyl group at the third position and a methoxy group at the fourth position of the naphthyl ring increase its activity. Also, physiologically and kinetically, these molecules are better than dynasore (Lee et al., 2010) (Fig. 5).

Macgregor et al. (2014) have synthesized naphthalimides, which inhibit the interaction between clathrin N-terminal domain and endocytic accessory proteins. One of 17,000 small molecules has been identified at the ChemBioNet Library. Further screening of various libraries showed 1,8- naphthol imides as being comparable inhibitors of clathrin. Refinement of the 4-aminobenzyl moiety gives a more active compound with better IC$_{50}$ values for clathrin inhibition. Macgregor et al. (2014) conclude that the bulky molecule fails to follow Lipinski's rule of five and is synthetically difficult to prepare. 1,8-Naphthyl anhydrides as starting materials are commercially available and were used to synthesize and screen 1,8-naphthalimides (Fig. 6). These compounds were found to have modest clathrin-inhibiting activity (Macgregor et al., 2014).

Mutations in LRRK2 is a frequent cause of autosomal familial PD. LRRK2 is involved in synaptic vesicle endocytosis

and exocytosis, and has been linked with DNM1, DNM2, and DNM3. Kavanagh et al. (2013) have reported amino pyrimidines GNE-7915 as brain-penetrating and nontoxic LRRK2 inhibitors. Kavanagh et al. (2013) have reported G-969 as an LRRK2 inhibitor with excellent potency (structure not disclosed). A number of compounds have been patented as LRRK2 inhibitors, but still there is a need to explore their detailed mechanism of action (Kavanagh et al., 2013).

McGeachie et al. (2013) have reported pyrimidine compounds as potent lipid-stimulated GTPase activity of both DNM1 and DNM2. They have reported pyrimidyn-7 as being the most potent compound in the dynamin inhibitor category. These compounds directly compete with GTP and thus block endocytosis (McGeachie et al., 2013). Robertson et al. (2012) have reported small-molecule rhodadyns as inhibitors of dynamin GTPase activity. From focused rhodadyn-based libraries, 13 compounds were found to be very potent for the inhibition of GTPase activity. These compounds block receptor-mediated endocytosis (RME) effectively, and two compounds, C10 and D10, have very good IC$_{50}$ values for RME (Robertson et al., 2012). Wang et al. (2012) have reported small-molecule inhibitors of tyrosine-(Y)-phosphorylation regulated kinase 1A, which gives hope for treatment of DS. Compounds have been found to be potent in in vitro cell-based assays. After performing structure-based virtual screening, six novel molecules have been reported as potent DYRKA1 inhibitors. The mechanism can be further explored to see the clinical benefits of these compounds in DS (Wang et al., 2012). Gordon et al. (2013) have reported a second-generation potent, indole-based dynamin GTPase
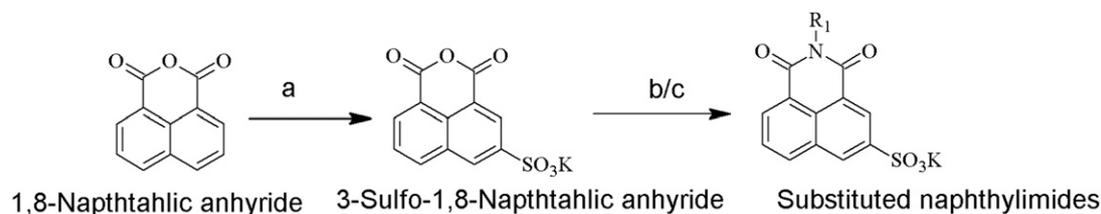
Fig. 6. General synthesis scheme for synthesis of 1, 8- naphthalimides. Reagents and conditions (a) fuming sulfuric acid 90°C, 90 min followed by saturated KCL, 4°C, 18 h; (b) RNH2, ethanol, reflux 18h or primary amine (0.1-1.5 equiv), 1 M lithium acetate,130 °C, 18-72 h.

inhibitor. Compound no. 24 is found to be the most active in the series (Gordon et al., 2013). Odell et al. (2010) have reported series of compounds such as pthaladyns based on the homology model for the GTP-binding domain of human DNM1. Pthaladyan-23 was found to be a potent inhibitor of DNM1-mediated synaptic vesicle endocytosis in brain synaptosomes (Odell et al., 2010). Hill et al. (2004, 2005, 2009) have reported amines and Dynoles for the inhibition of dynamin-mediated endocytosis. Dynole 34-2 has been

reported to be the most active inhibitor of RME and transferrin uptake (Hill et al., 2004, 2005, 2009). Takahashi et al. (2010) reported Sertraline as an inhibitor of dynamin GTPase activity and dynamin-dependent endocytosis. The authors have supported their hypothesis by performing cell line assays where Sertraline suppresses DNM1 as well as DNM2. Sertraline affects endocytosis via DNM2 (Yamada et al., 2009; Takahashi et al., 2010). Various recent reported dynamin ligand are given in Fig. 7.
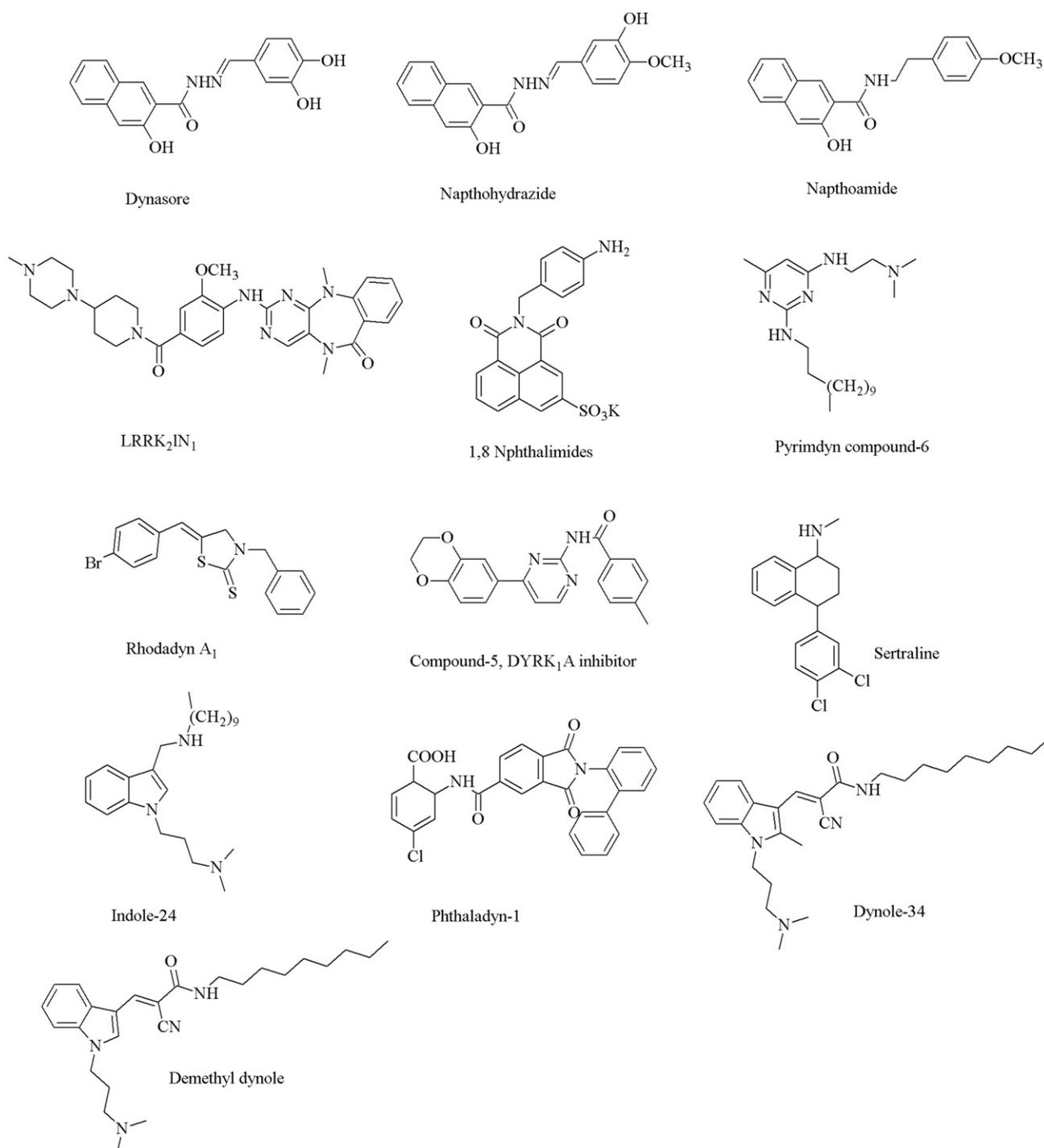
Fig. 7. Representative ligands of dynamin.

Conclusion and Discussion

Dynamin family members carry out a large number of functions in cell biology, including scission of vesicles, mitochondrial fusion, and tubulation during cytokinesis. The presence of GED, an MD, and cooperative GTPase activities are essential for biologic function. Understanding on a deep molecular level of dynamin interactions with their binding partners during vesicle biogenesis is still lacking. Though the role of dynamin has been linked with various disorders, such as AD, PD, HD, CMT, HF, schizophrenia, epilepsy, cancer, optic atrophy, DS, and osteoporosis, but the establishment of the pathophysiological role is still a challenge because animal models are not easily available. Considerable progress has been made for understanding the structural characterization of dynamins but still more details need to be explored. This review attempts to not only illustrate the mechanisms and roles of dynamin in the above-mentioned diseases, but also serves as a platform for a medicinal chemist to design novel dynamin ligands for various disorders.

Authorship Contributions

Wrote or contributed to the writing of manuscript: Singh, Jadhav, and Bhatt

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